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Analysis of pesticide residues in wine by solid-phase extraction and gas chromatography with electron capture and nitrogen–phosphorus detection☆

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Abstract

A feasible and reproducible method for multiresidue analysis of several common pesticides, of different polarities, in wine samples is proposed. The method combines a solid-phase extraction on polymeric cartridges eluted with ethyl acetate and a gas chromatographic determination using electron capture and nitrogen–phosphorus detection. To avoid the matrix effect, previous washing of the cartridges with a mixture of water–2-propanol (90:10) and further clean-up of the extract on Florisil cartridges, together with a calibration using spiked extracts, are recommended.

Keywords: Wine; Food analysis; Matrix effects; Extraction methods; Pesticides

1. Introduction

The extraction of pesticide residues in wine can be carried out by liquid–liquid extraction procedures, using solvents such as hexane [1], dichloromethane [2] or solvent mixtures as hexane–acetone [3–5], acetone–dichloromethane [6], and acetone–light petroleum [7]; however these methods are not free of drawbacks, such as the use of toxic and expensive solvents, the difficulty of automation, and the formation of emulsions. These problems can be overcome by using solid-phase extraction (SPE). So, its use has recently gained acceptance in the analysis of residues in wines, mainly with octadecylsilane (ODS) as stationary phase [8–12] although octylsilane [13] and charcoal [14] have also been used. In addition, SPE procedures have been coupled with gas chromatography for the analysis of pesticide residues in wine [15,16]. Other procedures, including the solid-phase microextraction, have also been used [17–19]. The SPE procedures on alkylsilane are usually adequate to analyze pesticides of low and medium polarity, but their application to more polar compounds (for instance malathion, parathion, pirifenox), or even pyrethroids, give frequently results with poor reproducibility and recovery; the addition of some salt to the wine sample to increase the ionic strength was tried to enhance their extraction [7,12].

The possible presence of residues of compounds

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of very different physico-chemical behaviour makes multiresidue extraction procedures necessary; for this reason, we have studied the SPE of 37 pesticides widely used in vineyards (including relatively polar compounds from different chemical families) testing two new polymeric phases, based on polystyrene–divinylbenzene (LiChrolut EN cartridges) and poly-(divinylbenzene–co-*N*-vinylpyrrolidone) (Oasis cartridges), and we have compared the results with those ones obtained using ODS cartridges. Furthermore, the application of these stationary phases to the analysis of three wine types (red, rose and white ones) is discussed.

At the same time, several clean-up modes have been assayed to reduce the influence of the matrix on the enhancement of the responses in the quantitative analysis; this effect has been reported in previous works on different types of samples, as well as on wines. This phenomenon is attributed to a higher transference of the analytes from the port injection to the chromatographic column while analyzing the extracts, in comparison with the transference achieved in the injection of the analytes solved in organic solvent. It also seems to be related to the state and maintenance of the liner and injection conditions [8,9,20–23].

2. Experimental

2.1. Material and chemicals

Pesticide standards were obtained from Promochem (Wesel, Germany) and Riedel–de Haën (Hannover, Germany). Residue analysis grade methanol, acetone, *n*-hexane, ethanol, 2-propanol, and ethyl acetate were provided by Labscan (Dublin, Ireland). Stock solutions of pesticides were made in acetone and *n*-hexane; dilutions were made in acetone. Sodium hydroxide of analysis grade was supplied by Panreac (Barcelona,Spain). Ultrapure water was obtained from a Milli-Q plus apparatus from Millipore (Bedford, MA, USA).

For SPE, Florisil and ODS 500 mg cartridges, and Oasis HLB 200 mg ones were supplied by Waters (Milford, MA, USA). LiChrolut EN 200 mg cartridges were obtained from Merck (Darmstadt, Germany).

2.2. Solid-phase extraction of pesticides in wine

A study about the extraction of the target-compounds by ODS, LiChrolut EN, and Oasis cartridges was done. The study was carried out on a 10 ml wine sample spiked with the pesticides at a concentration of 20 μg/l (in each). First, successive elution of 15 ml of methanol and 10 ml of water rinsed the cartridges. Next, the spiked sample was percolated through the cartridge at about 5 ml/min using a suction system. Then, 5 ml of a water–methanol (9:1) mixture was poured onto the cartridge, eluted and discarded, to clean-up the extract. The solvent was removed from the cartridges by passing nitrogen through it for about 45 min. The extract was eluted with 3 ml of an organic solvent after leaving the solid phase to soak for 4 min. Ethyl acetate and acetone were tested as eluents. The influence of both the amount of each pesticide in the wine (2–200 μg/l), and the kind of wine (white, red and rose) on the performance of the procedure were also investigated, using the stationary phase and the eluent that gave the best results (Oasis and ethyl acetate).

Two clean-up procedures were considered after establishing the extraction conditions. The experiments were made with 10 ml of wine spiked with 20 μg/l of each pesticide. Once the wine sample had been passed, mixtures of water–alcohol (90:10) were employed to rinse the cartridges; apart from water–methanol, mixtures of water–ethanol and water–2-propanol were also tested. Another clean-up solution assayed was 0.02 *M* NaOH.

The second procedure was based on Florisil cartridges, which were coupled to the extraction cartridges by Luer type connectors. Florisil cartridges were first conditioned by eluting 5 ml of ethyl acetate and air-dried for 5 min. Then, the cartridges were coupled to the extraction ones, and the ethyl acetate (3 ml) that elute the pesticides from the Oasis cartridge was let down directly on the Florisil one. This eluate was collected and, later, analyzed by GC.

2.3. Determination by GC–electron-capture detection (ECD)

A Hewlett-Packard (Avondale, PA, USA) 5890 gas chromatograph equipped with an HP7673 auto-sampler, an electron-capture detector, and two columns

were employed. A 30 m×0.25 mm capillary column coated with a 0.25 μm thick film of 35% phenylmethylpolysiloxane (HP-35) from Hewlett-Packard was used in combination with the following oven temperature program: initial temperature 50°C, held for 1 min, 40°C/min ramp to 190°C, then 0.6°C/min ramp to 242°C, and finally 70°C/min ramp to 300°C, held for 30 min. The second column, a 60 m×0.25 mm capillary column coated with a 0.25 μm thick film of 50% phenylmethylpolysiloxane (CPSIL 24CB) from Chrompack (Middelburg, Netherlands) was used in combination with this oven temperature program: initial temperature 50°C, held for 1 min, 10°C/min ramp to 140°C, and finally 2.3°C/min ramp to 275°C, held for 82 min.

The carrier gas (He) flow-rate was 0.7 ml/min, measured at 50°C. Splitless injection of a 2 μl volume was carried out at 225°C with the purge valve on at 1 min. Argon–methane (90:10) was used as an auxiliary gas for ECD. Detector temperatures were 300°C.

2.4. Determination by GC with nitrogen–phosphorus detection (NPD)

A Perkin-Elmer Autosystem gas chromatograph equipped with an auto-sampler, a nitrogen–phosphorus detector, and a 60 m×0.25 mm capillary column coated with a 0.25 μm thick film of 5% phenylmethylpolysiloxane (CPSIL-5CB) from Chrompack (Surrey, UK) was used. The oven temperature program was as follows: initial temperature 50°C, held for 1 min, 15°C/min ramp to 200°C, and finally 1°C/min ramp to 275°C, held for 30 min. The carrier gas (He) flow-rate was 0.7 ml/min, measured at 50°C. Splitless injection of a 2 μl volume was carried out at 200°C with the purge valve on at 1 min. Hydrogen, air and helium were used as auxiliary gases for NPD. Detector temperature was 300°C.

3. Results and discussion

3.1. Pesticide extraction

In Table 1, the recoveries obtained with the three types of cartridges and eluting with acetone or ethyl acetate are summarized. As can be seen, for the compounds detected by ECD, the recoveries with the ODS cartridges eluted with acetone are higher than 100%, except for endosulfan, lindane and pyrethroids; the recoveries obtained using ethyl acetate were also higher for all the compounds, even bigger than those ones obtained with acetone.

The elution of Oasis cartridges with acetone did not allow the quantitative extraction of compounds such as bromopropilate or the pyrethroids; on the other hand, using ethyl acetate all the compounds were extracted with similar percentages to those obtained with ODS cartridges. The same happened when LiChrolut EN cartridges were used. For the three phases tested the matrix effects were bigger when ethyl acetate was used instead of acetone.

When more polar compounds were determined by GC–NPD, the extraction with ODS cartridges was not suitable. Oasis and LiChrolut EN allowed the extraction of these compounds and the quantitative results were also affected by the presence of co-extracted substances from the wine matrix; this matrix effect was more important when ethyl acetate was chosen as eluent, as can be observed in Table 1. It is interesting to remark that omethoate, pesticide whose solubility in water usually makes its extraction in solid phase difficult, was retained in LiChrolut EN cartridges.

The chromatograms obtained by GC–ECD were also different, so the obtained ones from the use of ODS cartridges were much cleaner compared with those obtained after using the other two phases, which had irregular baselines, a lot of co-extracted compounds and a bigger front. As it happened when analyzing must [8], the recoveries increased as the chromatogram got more complicated. That can be observed examining both Table 1 and Fig. 1. Similar consideration must be made if the data achieved on the same cartridge by eluting acetone or ethyl acetate are compared. In another work devoted to the analysis of wines, recoveries enhanced up to 300–400% were also reported for some less stable pesticides at a concentration of 20 μg/l [9].

Chromatograms obtained with GC–NPD were simpler because of the higher selectivity of this detector (Fig. 1d), except for those belonging to LiChrolut EN cartridges where the number of co-extractives was higher.

Table 1
Recoveries (in %) obtained in the extraction of 10 ml of red wine samples spiked with 20 μg/l of each pesticide, using different cartridges and eluents ($n=5$)

No.	Compound	Cartridge/eluent					
		ODS/ Acetone	ODS/ethyl acetate	LiChrolut EN/ acetone	LiChrolut EN/ ethyl acetate	Oasis/ acetone	Oasis/ethyl acetate
Compounds determined by GC–ECD							
1	Lindane	86	109	88	83	42	102
2	Vinclozoline	141	174	129	228	59	150
3	Chlorpyrifos methyl	132	181	141	160	247	160
4	Triadimefon	786	328	264	496	81	324
5	Dichlofluanid	112	164	120	107	76	144
6	4,4′-Dichlorobenzophenone	352	428	264	318	121	266
7	Penconazole	262	434	350	430	166	530
8	Triadimenol	230	458	306	334	354	518
9	Pirifenox 1	214	306	226	212	130	380
10	Procymidone	192	370	364	412	<LOC	448
11	Endosulfan A	63	83	47	71	<LOC	53
12	Pirifenox 2	191	278	212	248	306	210
13	Captan	206	282	226	248	1738	266
14	Tetrachlorvinphos	230	334	310	290	95	322
15	Endosulfan B	94	131	78	115	<LOC	119
16	Endosulfan sulfate	91	160	633	130	18	156
17	Nuarimol	147	282	187	230	60	354
18	Bromopropilate	180	266	145	234	19	234
19	Captafol	272	394	344	384	182	404
20	Tetradifon	244	330	240	382	73	324
21	Fenarimol	170	298	222	251	81	340
22	Cypermethrin 1	212	250	236	244	<LOD	250
	Cypermethrin 2	210	254	234	263	<LOD	270
	Cypermethrin 3	182	248	187	246	<LOD	258
23	Fenvalerate 1	115	164	136	145	<LOD	172
	Fenvalerate 2	134	186	154	169	<LOD	214
24	Deltamethrin	169	220	184	199	<LOD	216
Compounds determined by GC–NPD							
1	Dichlorvos	<LOD	45	347	263	32	488
	Omethoate	<LOD	<LOD	83	65	<LOD	<LOD
2	Simazine	42	69	228	596	144	379
3	Pyrimethanil	21	47	150	214	86	225
4	Diazinon	34	57	183	245	67	263
5	Parathion methyl	34	65	283	322	80	324
6	Fenitrothion	40	45	152	171	69	206
7	Malathion	37	29	290	308	83	355
8	Cyprodinil	51	49	204	287	94	287
9	Mepanipyrim	25	34	281	302	72	258
10	Kresosim-methyl	76	84	207	241	49	277
11	Ethion	65	93	192	195	54	199
12	Benalaxil	70	64	185	204	108	157
13	Iprodione	39	48	353	254	190	384

After the mentioned experiments, the extraction using Oasis cartridges eluted with ethyl acetate was chosen as the best option because it allowed to extract nearly all the compounds independent of their polarities, giving chromatograms clean enough to identify and determine the residues. The typical ODS

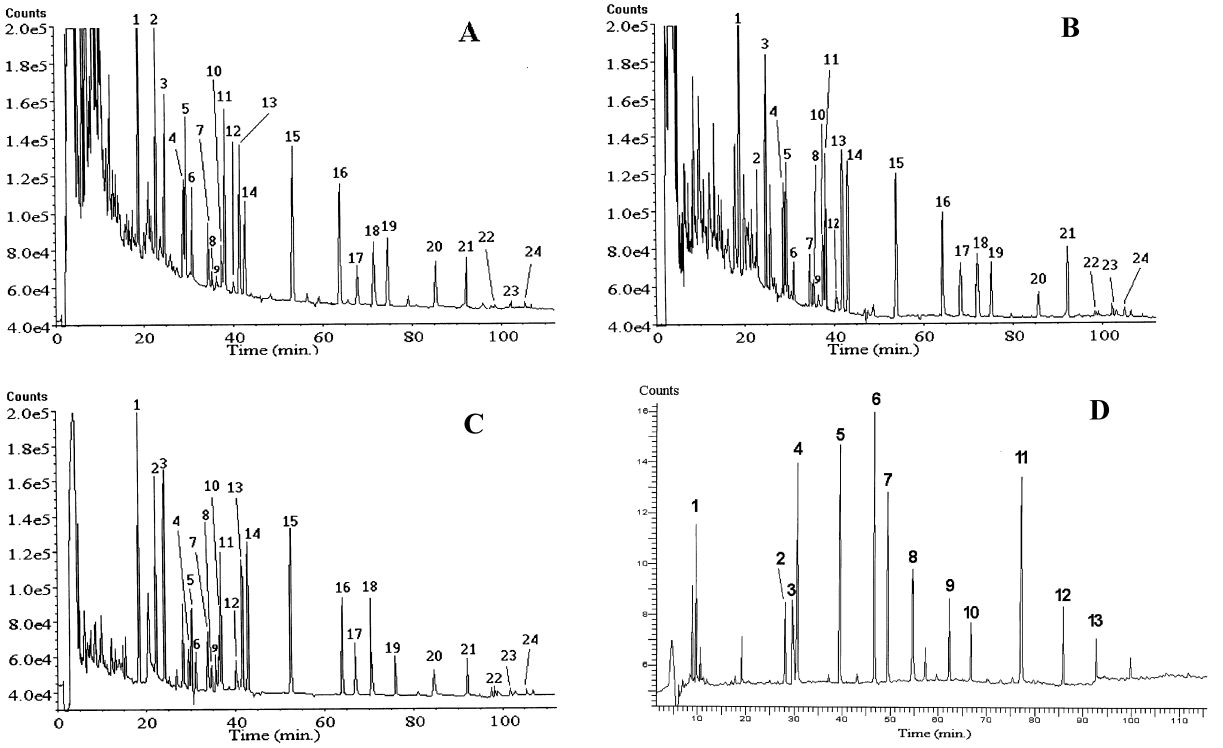

Fig. 1. Chromatograms obtained from extracts of red wine spiked with 20 μg/l (ECD) and 200 μg/l (NPD) of each pesticide. (A) Extraction on LiChrolut EN cartridges; elution with ethyl acetate; ECD. (B) Extraction on Oasis cartridges; elution with ethyl acetate; ECD. (C) Extraction on ODS cartridges; elution with ethyl acetate; ECD. (D) Extraction on Oasis cartridges; elution with ethyl acetate; NPD. See Table 1 for peak identification.

cartridges showed a low retention for organophosphorus and organonitrogen compounds.

3.2. Matrix effects

The matrix effect became lower as the concentration of residues, in the spiked samples, increased from 2 μg/l to 200 μg/l, for example, the recovery for a 2 μg/l spiked sample was around 25% larger than that obtained from a sample spiked at 20 μg/l level.

The selected procedure was applied to different samples of red, rose and white wines from Rueda (guarantee of origin and quality) in order to check the possible influence of the matrix, the results for some samples are shown in Table 2. Recoveries were quite similar, as well as chromatograms. The average reproducibility was 4–10%. However, in the above-mentioned work [9], the recoveries were clearly higher for the white wines than for the red wines.

Another experiment was carried out by changing the insert and the capillary column (replacing the HP-35 column for a CPSIL 24CB one) in the GC–ECD system. The recoveries obtained then were also very high in several cases, but of different magnitude, for example for the pyrethroids, they were lower than when using the first column. That could be due to the different state of the injection port in the chromatograph.

3.3. Clean-up of the extracts

Trying to reduce the matrix effect and to simplify the chromatograms, several clean-up procedures were assayed. The first attempt was the use of cartridges washed with 0.02 *M* NaOH, dried and then eluted with ethyl acetate. The chromatograms

Table 2
Recoveries (in %) obtained in the extraction of 10 ml of wine spiked with 20 μg/l of each pesticide after eluting the sample on Oasis cartridges and collecting the extract with 3 ml of ethyl acetate (n=5)

No.	Compound	Wine			
		Red 1	Red 2	Rose	White
Compounds determined by GC–ECD					
1	3,5-Dichloroaniline	228	234	327	363
2	Lindane	74	84	87	79
3	Vinclozoline	105	92	101	99
4	Chlorotalonil	98	82	100	114
5	Chlorpyrifos methyl	122	104	110	95
6	Triadimefon	142	138	123	133
7	Dichlofluanid	101	99	103	101
8	4,4′-Dichlorobenzophenone	140	121	145	124
9	Penconazole	141	140	138	145
–	Triadimenol	224	202	222	195
–	Pirifenox 1	189	191	201	185
10	Procymidone	168	115	110	105
11	Endosulfan A	71	68	59	44
–	Pirifenox 2	195	205	210	199
12	Tetrachlorvinphos	149	156	154	164
13	Captan	39	141	142	152
14	Endosulfan B	77	71	64	47
15	Endosulfan sulfate	94	90	87	73
16	Nuarimol	101	108	110	104
–	Bromopropilate	156	148	150	149
17	Captafol	321	300	295	310
18	Tetradifon	79	89	89	55
19	Fenarimol	66	98	98	65
20	Cypermethrin 1	22	30	30	21
	Cypermethrin 2	18	33	33	22
	Cypermethrin 3	27	34	34	19
21	Fenvalerate 1	25	34	34	17
	Fenvalerate 2	25	41	40	20
–	Deltamethrin	21	41	45	35
Compounds determined by GC–NPD					
	Dichlorvos	468	440	458	439
	Simazine	357	361	387	384
	Pyrimethanil	230	245	228	239
	Diazinon	265	281	270	254
	Parathion methyl	329	318	341	358
	Fenitrohtion	200	213	220	198
	Malathion	342	359	341	367
	Cyprodinil	301	294	278	288
	Mepanipyrim	267	259	248	263
	Kresosim-methyl	281	298	300	315
	Ethion	214	247	209	185
	Benalaxyl	171	151	160	163
	Iprodione	372	351	342	369

obtained were very clean, but several compounds were partially destroyed or eluted by the NaOH, mainly for low residue concentration. Thus the procedure could be useful for samples with contents near 200 μg/l but not for concentrations of 20 μg/l or lower.

Other solutions were also assayed to wash the cartridges, mainly water–alcohol mixtures. Using the mixture water–2-propanol (90:10) the chromatograms obtained had less front and less background and, for certain compounds, the matrix effects were also reduced, in comparison with those ones obtained for the mixtures water–methanol or water–ethanol.

The best procedure turned out to be the use of Florisil cartridges, because the matrix effect was notably diminished. For example, in captan analysis, the recovery of 205% (clean-up with water–2-propanol) was reduced to 130%. In Fig. 2, some examples are shown. A reduction in the background was observed when using ECD, while the chromatograms were similar in NPD.

3.4. Sample treatment proposed procedure

As a consequence of the experiments made, we have selected, to analyze residues in wine samples by gas chromatography, a procedure that combines the washing of the cartridges with the mixture water–2-propanol (90:10) and a clean-up on Florisil cartridges. It is as follows: a 10 ml wine sample was passed through an Oasis cartridge that afterwards is washed with 5 ml of a mixture water–*n*-propanol (9:1), then the cartridge is air-dried for 45 min, next 3 ml of ethyl acetate are used to elute the residues after a soaking time of 4 min. This eluate is flowed through the Florisil cartridge, coupled in series, and an aliquot injected into the chromatograph.

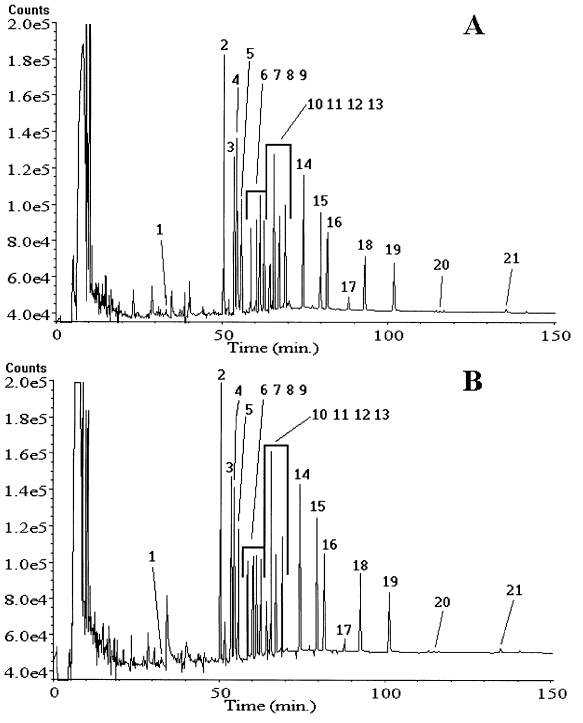

Fig. 2. Chromatograms obtained for a sample of red wine spiked with 20 μg/l of each pesticide and extracted with Oasis cartridges (elution with ethyl acetate). (A) Clean-up with water–2-propanol (9:1). (B) Clean-up with Florisil. See Table 2 for peak identification.

3.5. Correction of the matrix effects

Applying the proposed procedure to spiked wine samples, it could be observed that although the matrix effects had been notably reduced, they still gave recovery values that did not allow an adequate quantitation of residues. For this reason, we used a matrix-standard calibration: wines samples were spiked with variable amounts of pesticides (whose concentrations can be seen in Table 4) and subjected to the same treatment as the samples. The extracts obtained from the spiked wines were considered as standards to obtain the calibration graph. The recoveries obtained were near 100% and the relative standard deviations ranging between 3 and 15%. If the polychlorinated biphenyl (PCB) 52 in GC–ECD and myclobutanil in GC–NPD were used as internal standards, the reproducibility of the procedure was better (RSDs between 3 and 8%). In Table 3, the results obtained by calibrating with the internal standard method are summarized. In Table 4, the linearity intervals (referred to residue determination in wine samples), the correlation coefficients and the detection limits (calculated experimentally from a signal-to-noise ratio of 5) are presented.

4. Conclusions

The use of polymeric phases, as Oasis, with ethyl acetate as eluent, in GC multiresidue analysis of pesticides of very different polarity, in wine samples, turns out to be a good alternative for the traditional ODS extraction.

Anomalous high recoveries, similar for different

Table 3
Recoveries (in %) obtained in the extraction of wine samples spiked with 20 μg/l of each pesticide after applying the proposed procedure and using a calibration graph made with extracts from spiked red wine ($n=5$)

Compound	Wine			
	Red 1	Red 2	Rose	White
Compounds determined by GC–ECD				
3,5-Dichloroaniline	90	108	84	104
Lindane	92	95	84	76
Vinclozoline	91	110	100	119
Chlorotalonil	93	96	83	93
Chlorpyrifos methyl	95	100	101	110
Triadimefon	109	104	95	109
Dichlofluanid	82	81	94	102
4,4′-Dichlorobenzophenone	104	100	94	90
Penconazole	108	99	100	100
Triadimenol	106	98	91	99
Pirifenox 1	100	100	83	92
Procymidone	106	101	90	88
Endosulfan A	89	93	95	84
Pirifenox 2	95	97	90	90
Tetrachlorvinphos	113	112	105	107
Captan	97	90	95	96
Endosulfan B	88	95	89	80
Endosulfan sulfate	83	80	85	86
Nuarimol	102	101	102	112
Bromopropilate	100	89	90	97
Captafol	101	95	104	92
Tetradifon	92	99	87	81
Fenarimol	106	104	104	120
Cypermethrin 1	85	100	108	109
Cypermethrin 2	88	102	107	103
Cypermethrin 3	90	106	101	103
Fenvalerate 1	92	107	99	95
Fenvalerate 2	94	101	98	96
Deltamethrin	94	95	100	100
Compounds determined by GC–NPD				
Dichlorvos	104	99	101	110
Simazine	110	102	89	91
Pyrimethanil	98	95	94	99
Diazinon	104	97	95	103
Parathion methyl	103	107	94	99
Fenitrothion	94	92	99	104
Malathion	107	104	110	101
Cyprodinil	105	98	99	107
Mepanipyrim	104	102	97	98
Kresosim-methyl	91	104	95	100
Ethion	100	108	117	106
Benalaxyl	94	92	99	104
Iprodione	104	114	93	97

kinds of wine, appear and are more accused for lower residue concentrations. This effect is put down to the influence of the substances co-extracted from the matrix in the injection port of the chromatographic system, and it is also related to its maintenance and fungible material used.

Table 4
Linearity, coefficient of correlation (r^2), and detection limits (LODs) obtained after applying the proposed procedure and using a calibration graph (internal standard method) made with extracts of spiked samples ($n=5$)

Compound	Linearity (μg/l)	r^2	LOD (μg/l)
Compounds determined by GC–ECD			
3,5-Dichloroaniline	50–500	0.996	10
Lindane	0.5–500	0.9999	0.1
Vinclozoline	0.5–500	0.998	0.1
Chlorotalonil	5–500	0.993	1
Chlorpyrifos methyl	25–500	0.98	3
Triadimefon	5–500	0.995	0.2
Dichlofluanid	1–500	0.999	0.2
4,4′-Dichlorobenzophenone	0.5–500	0.990	0.04
Penconazole	1–500	0.998	0.2
Triadimenol	50–500	0.990	7
Pirifenox 1	50–500	0.990	12
Procymidone	10–500	0.993	2
Endosulfan A	0.5–500	0.995	0.02
Pirifenox 2	50–500	0.98	12
Tetrachlorvinphos	0.5–500	0.998	0.1
Captan	10–500	0.997	0.2
Endosulfan B	0.5–500	0.996	0.1
Endosulfan sulfate	0.1–500	0.998	0.01
Nuarimol	0.5–500	0.995	0.05
Bromopropilate	0.5–500	0.994	0.05
Captafol	0.5–500	0.997	5
Tetradifon	0.2–500	0.991	0.02
Fenarimol	0.5–500	0.995	0.1
Cypermethrin 1	30–500	0.9999	6
Cypermethrin 2	30–500	0.9999	6
Cypermethrin 3	30–500	0.9999	6
Fenvalerate 1	25–500	0.9990	4
Fenvalerate 2	25–500	0.9990	4
Deltamethrin	25–500	0.9994	2
Compounds determined by GC–NPD			
Dichlorvos	5–500	0.991	1
Simazine	25–500	0.992	3
Pyrimethanil	10–500	0.993	5
Diazinon	1–500	0.991	0.4
Parathion methyl	0.5–500	0.997	0.1
Fenitrothion	1–500	0.996	0.2
Malathion	0.5–500	0.9991	0.1
Cyprodinil	25–500	0.992	10
Mepanipyrim	25–500	0.994	4
Kresosim-methyl	10–500	0.998	4
Ethion	0.01–500	0.994	0.05
Benalaxyl	25–500	0.996	5
Iprodione	25–500	0.98	5

The clean-up with a water–2-propanol (90:10) mixture and Florisil cartridges contributes to reducing the matrix effects, which can only be avoided by making a calibration with extracts of spiked wine samples. That calibration can be useful to analyze different kinds of wines.

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